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Cladorrhinum yunnanense sp. nov. from China

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Abstract—A new asexual fungus, *Cladorrhinum yunnanense*, isolated from unidentified fallen leaves of a broadleaf tree in Yunnan, China, is described by morphological characters and phylogenetic analyses of DNA sequence data from three loci (ITS, LSU and TUB).

Key words—*Lasiosphaeriaceae*, mitosporic fungi, *Sordariales*, taxonomy

Introduction

Cladorrhinum Sacc. & Marchal was established in 1885 with *C. foecundissimum* Sacc. & Marchal as the type species (Marchal 1885). The genus is characterized by fast-growing colonies with pale to dark greenish, greyish, or brownish pigmentation, and conidiophores typically comprising intercalary phialides with lateral openings (pleurophialides), usually with a flaring collarette. The conidia are one-celled, hyaline or subhyaline, smooth-walled, commonly globose to dacryoid, and arranged in slimy masses. *Bahupaathra* Subram. & Lodha is synonymised with the earlier described *Cladorrhinum* (Mouchacca & Gams 1993).

[#] These authors contributed equally to this work.

Mouchacca & Gams (1993), who revised *Cladorrhinum*, accepted five species: *C. foecundissimum*, *C. brunnescens* W. Gams, *C. bulbillosum* W. Gams & Mouch., *C. phialophoroides* Mouch. & W. Gams, and *C. samala* (Subram. & Lodha) W. Gams & Mouch. Subsequently added to the genus were *C. flexuosum* Madrid & al., *C. microsclerotigenum* Madrid & al., *C. australe* Gasoni, *C. globisporum* Z.F. Zhang & L. Cai, *C. hyalocarpum* (Arx) X. Wei Wang & Houbraken, and *C. intermedium* (Stchigel & Guarro) X. Wei Wang & Houbraken (Madrid & al. 2011, Carmaran & al. 2015, Zhang & al. 2017, Wang & al. 2019). Wang & al. (2019) recently transferred *C. bulbillosum* to *Podospora* Ces. and *C. phialophoroides* to *Triangularia* Boedijn.

Fungal diversity in China is high but still mostly unexplored. Several new species from Yunnan Province have been previously described (Xia & al. 2012, 2014, 2016; Ma & al. 2014 a,b,c; Zhang & al. 2016; Guo & al. 2019; Zheng & al. 2019, 2020). During our ongoing survey of anamorphic fungi associated with plant leaves in tropical and subtropical forests of southern China, we collected a new species representing *Cladorrhinum.*

Materials & methods

Isolates and Morphological analysis

Diseased leaves were collected from an unidentified plant in Xishuangbanna, Yunnan Province, China. Tissue pieces (5 × 5 mm) were taken from the margin of leaf lesions and surface-sterilized by immersing in 75% ethanol solution for 1 min, 5% sodium hypochlorite solution for 30 s, and then rinsing three times in sterile distilled water for 1 min. The pieces were dried with sterilized paper towels and then placed on potato dextrose agar (PDA) (Cai & al. 2009). All the PDA plates were incubated at room temperature 25 °C for 2–4 d, and from which the peripheries of the colonies were picked out and inoculated onto new PDA plates. Colonies were photographed after 7 d and 15 d with a Powershot G7X mark II digital camera. Micromorphological characters were observed using an Olympus SZX10 stereomicroscope and Olympus BX53 microscope, both fitted with Olympus DP80 high definition colour digital cameras. All fungal strains were stored in 10% sterilized glycerin at 4 °C for further studies. The specimens are deposited in the Herbarium of Plant Pathology, Shandong Agricultural University, Taian, China (HSAUP). Ex-type cultures are deposited in the Shandong Agricultural University Culture Collection, Taian, China (SAUCC).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from colonies grown on PDA, using the CTAB method (Doyle & Doyle 1990). The primer pair ITS1/ITS4 (White & al. 1990) was

TABLE 1. *Cladorrhinum* and related strains included in the phylogenetic analyses.

SPECIES NAME	CULTURE ACC. NO.	GENBANK ACCESSION NO.		
		ITS	LSU	TUB
Cercophora ambigua	CBS 215.60	AY999137	AY999114	AY999147
Cercophora appalachianensis	HKUCC3711	AF177155	—	—
Cercophora areolata	UAMH7495	AY587911	AY587936	AY600252
Cercophora caudata	CBS 606.72	AY999135	MH872289	AY999151
Cercophora grandiuscula	CBS 120013^{T}	GQ922544	KF557693	—
Cercophora samala	CBS 109.93^{T}	AY999134	AY999111	AY999140
Cercophora sulphurella	SMH2531	AY587913	AY587938	AY600254
Cladorrhinum australe	INTA-AR 108	KT321073	KT312986	KT291711
	INTA-AR 110	KT321074	KT312987	KT291712
Cladorrhinum brunnescens	CBS 643.75^{T}	NR137152	FR692346	—
Cladorrhinum bulbillosum	INTA-AR 102	KT321068	KT312982	KT291706
	CBS 304.90^{T}	NR077199	FR692339	KT291720
Cladorrhinum flexuosum	FMR 10415	NR154757	NG058773	—
Cladorrhinum foecundissimum	BCCM 6980	KT321080	KT312993	KT291721
	CBS 180.66^{T}	MH858767	FR692343	KT291717
Cladorrhinum globisporum	CGMCC3.17921^{T}	KU746680	KU746726	KU746771
	CGMCC3.17922	KU746679	KU746725	KU746772
Cladorrhinum hyalocarpum	CBS 322.70^{T}	MK926857	MK926857	MK926957
	CBS 102198	MK926858	MK926858	MK926958
Cladorrhinum intermedium	CBS 433.96^{T}	MK926859	MK926859	MK926959
	CBS 100257	MK926860	MK926860	MK926960
Cladorrhinum microsclerotigenum	CBS 290.75^{T}	NR154758	FR692342	—
Cladorrhinum phialophoroides	CBS 301.90^{T}	NR077198	FR692344	KT291718
Cladorrhinum samala	CBS 302.90	KT321079	KT312992	KT291719
	INTA-AR 168	KT321078	KT312991	KT291716
Cladorrhinum yunnanense	**SAUCC 0525** $^{[HT]}$	**MN956794**	**MN956785**	**MT376683**
	SAUCC 0791	**MN956795**	**MN956786**	—
Phialophora brevicollaris	CBS 126.74^{T}	NR121471	—	GU727570
Podospora cochleariformis	CBS 249.71	MH860099	AY999098	AY999145
Podospora cupiformis	CBS 246.71^{T}	MH860098	AY999102	AY999149
Podospora curvicolla	IFO 8548	AY999122	AY999099	AY999148
Podospora decipiens	CBS 258.69	KX171946	AY780073	AY780130
Podospora didyma	CBS 232.78	AY999127	AY999100	AY999142
Podospora fibrinocaudata	CBS 315.91^{T}	MH862255	MK926844	MK926944
Podospora fimiseda	CBS 990.96	KX348039	AY346296	AY780133
Podospora intestinacea	CBS 113106	AY999121	AY999104	AY999152

used to amplify the rDNA internal transcribed spacer (ITS) region following White & al. (1990). The primer pair LR0R/LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994) was used to amplify a partial fragment of the large subunit (LSU) gene. The primer pair Bt2a/Bt2b (Glass & Donaldson 1995) was used to amplify the beta-tubulin (TUB) gene.

The DNA was amplified using an Eppendorf Master Thermocycler in 25 µL reaction volumes containing 12.5 µL Vazyme Green Taq Mix, 1 µL of each Biosune forward and reverse primer (10 µM), and 1 µL template genomic DNA adjusted with distilled deionized water to a total 25 µL volume. PCR parameters were 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C (ITS, LSU) or 60 °C (TUB) for 30 s, extension at 72 °C for 1 min, and a final elongation step at 72 °C for 10 min. PCR products were estimated visually by staining with GelRed after 1% agarose gel electrophoresis. Sequencing was done bi-directionally, conducted by the Biosune Company Limited. Consensus sequences were obtained using MEGA v. 7 (Kumar & al. 2016). All sequences generated in this study were deposited in GenBank (TABLE 1).

Sequence alignment and phylogenetic analysis

The quality of our amplified nucleotide sequences was checked and combined by MEGA v. 7 (Kumar & al. 2016), and reference sequences were retrieved from the National Center for Biotechnology Information (NCBI), based on recent publications on *Cladorrhinum* (Madrid & al. 2011, Carmarán & al. 2015, Zhang & al. 2017). Sequences were aligned using MAFFT v. 7.310 (Katoh & al. 2019; http://mafft.cbrc.jp/alignment/server/index.html) and manually corrected using MEGA v. 7.

The combined gene regions were phylogenetically analysed using Maximum-Likelihood (ML) and Bayesian Inference (BI) methods. RaxML (v. 8.2.9) and Bayesian analyses (MrBayes v. 3.2.6) were run on the CIPRES Science Gateway portal (Miller & al. 2012). Evolutionary models were calculated using MrModelTest v. 2.3 (Nylander 2004) selecting the best-fit model for each data partition according to the Akaike criterion. For ML analyses default parameters were used and bootstrap support (BS) used the rapid bootstrapping algorithm with the automatic halt option. Bayesian analyses included two parallel runs of 5,000,000 generations, with the stop rule option and a sampling frequency set to each 1000 generations. The 50% majority rule consensus trees and posterior probability (PP) values were calculated after discarding the first 25% of the samples as burn-in. The resulting trees were plotted using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree) and edited with Adobe Illustrator CS v. 5. The individual gene datasets were assessed for incongruence before being concatenated by checking their individual phylogenies for conflicts between clades with significant ML and BI support (Mason-Gamer & Kellogg 1996, Wiens 1998).

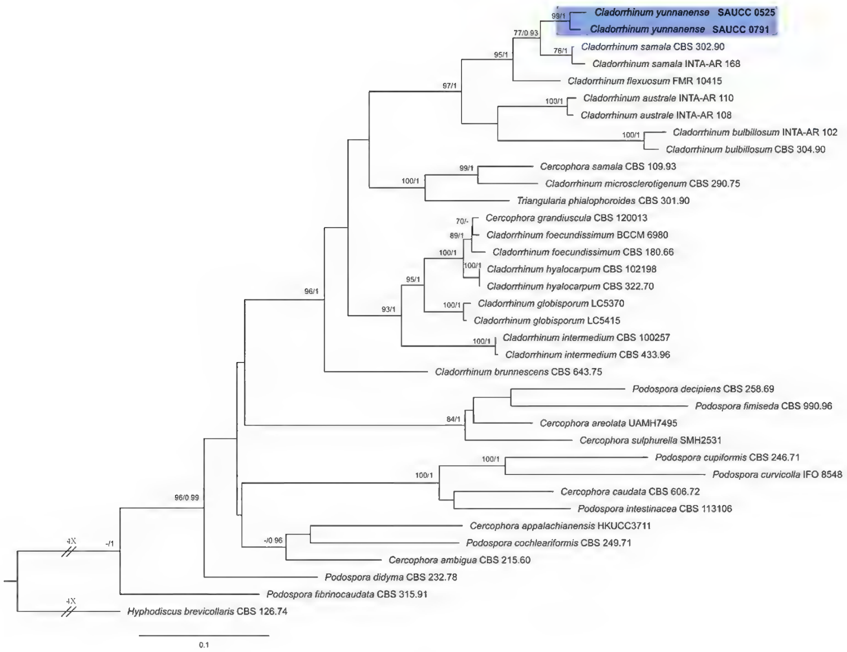
99/1
Cladorrhinum yunnanense SAUCC 0525
Cladorrhinum yunnanense SAUCC 0791
77/0.93
76/1
Cladorrhinum samala CBS 302.90
Cladorrhinum samala INTA-AR 168
95/1
Cladorrhinum flexuosum FMR 10415
97/1
100/1
Cladorrhinum australe INTA-AR 110
Cladorrhinum australe INTA-AR 108
100/1
Cladorrhinum bulbillosum INTA-AR 102
Cladorrhinum bulbillosum CBS 304.90
99/1
Cercophora samala CBS 109.93
100/1
Cladorrhinum microsclerotigenum CBS 290.75
Triangularia phialophoroides CBS 301.90
70/-
Cercophora grandiuscula CBS 120013
89/1
Cladorrhinum foecundissimum BCCM 6980
100/1
Cladorrhinum foecundissimum CBS 180.66
100/1
Cladorrhinum hyalocarpum CBS 102198
95/1
Cladorrhinum hyalocarpum CBS 322.70
96/1
100/1
Cladorrhinum globisporum LC5370
93/1
Cladorrhinum globisporum LC5415
100/1
Cladorrhinum intermedium CBS 100257
Cladorrhinum intermedium CBS 433.96
Cladorrhinum brunnescens CBS 643.75
Podospora decipiens CBS 258.69
Podospora fimiseda CBS 990.96
84/1
Cercophora areolata UAMH7495
Cercophora sulphurella SMH2531
100/1
Podospora cupiformis CBS 246.71
100/1
Podospora curvicolla IFO 8548
96/0.99
Cercophora caudata CBS 606.72
Podospora intestinacea CBS 113106
Cercophora appalachianensis HKUCC3711
4X
-/1
-/0.96
Podospora cochleariformis CBS 249.71
Cercophora ambigua CBS 215.60
Podospora didyma CBS 232.78
Podospora fibrinocaudata CBS 315.91
4X
Hyphodiscus brevicollaris CBS 126.74
0.1

FIG. 1. The ML consensus tree inferred from the combined ITS, LSU and TUB sequence alignment. Branches are labeled with Maximum-Likelihood bootstrap support ≥75% and Bayesian posterior probabilities ≥0.95. The tree is rooted to *Hyphodiscus brevicollaris* (CBS 126.74). The scale bar indicates 0.1 expected changes per site.

Phylogenetic results

The dataset comprised 36 taxa representing 28 species including *Hyphodiscus brevicollaris* (CBS 126.74) as the outgroup. The final alignment comprised a total of 2101 characters of the combined ITS, LSU, and TUB including gaps: ITS 1–545; LSU, 546–1357; and TUB, 1358–2101. Of these characters, 1315 were constant, 221 parsimony-uninformative, and 565 parsimony-informative. For ML and BI analyses, GTR+I+G for ITS and LSU, and GTR+G for TUB were selected and incorporated into the analyses. As the topology of ML tree confirmed the tree topologies obtained from the BI analyses, only the ML tree is presented (FIG. 1). In this tree, our strains formed a distinct clade and grouped together with *Cladorrhinum samala* and *C. flexuosum* with good support (BS = 95%, PP = 1). Therefore, we determined that our strains belonged to a novel species of *Cladorrhinum*.

Taxonomy

Cladorrhinum yunnanense Y. Pan, J.W. Xia, X.G. Zhang & Z. Li, **sp. nov.** FIG. 2

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Differs from all other *Cladorrhinum* spp. by its blastic conidiogenous cell.

TYPE: China, Yunnan Province, Xishuangbanna, on unidentified fallen leaf of a broadleaf tree, 16 Apr. 2019, Y. Pan (**Holotype**, HSAUP 0525; ex-type living culture SAUCC 0525).

ETYMOLOGY: in reference to the province containing the type locality.

COLONIES on PDA pale grey to dark grey, cottony to fluffy, flat to slightly plicate, margin entire. Reverse pale brown to brown. Culture on OA white to grayish, margin entire, aerial mycelia sparse. Reverse white. Culture on MEA white to yellowish-brown, cottony to fluffy, margin filiform. Reverse white to yellowish. VEGETATIVE HYPHAE hyaline, branched, septate, thin-walled, slightly rough, 1.5–4 µm diam. MICROSCLEROTIA not observed. SPORODOCHIUM forming on PDA within 30 d or longer, margin round, black, scattered over entire colony. CONIDIOPHORES single, unbranched, erect, straight or slightly flexuous, cylindrical, smooth, thick-walled, hyaline at the base, pale brown to brown in the upper part, 2–4-septate, 40–60 × 2–4 µm. CONIDIOGENOUS CELL polyblastic, integrated, terminal, cylindrical, pale brown to brown, swollen at the apex, 15–25 × 2–4 µm. CONIDIA pale brown, smooth, thick-walled, oval to ellipsoid, aseptate, 6–10 × 2–4 µm, guttulate, aggregated in slimy head.

ADDITIONAL SPECIMEN EXAMINED: **CHINA, YUNNAN PROVINCE, Xishuangbanna,** on unidentified fallen leaf of a broadleaf tree, 16 Apr. 2019, Y. Pan (HSAUP 0791; living culture SAUCC 0791).

COMMENTS—According to Madrid & al. (2011) regarding convergent morphology in *Cladorrhinum*, characterization of any potentially new species within this genus should be included in the phylogenetic analyses. Based on a megablast search of GenBank nucleotide sequence database, the closest hit using the ITS sequence was *Cladorrhinum* sp. (strain RS_94, GenBank MK379585.1; Identities = 481/489 (98%), 3 gaps (0%)); the closest hit using the LSU sequence was *Cercophora costaricensis* (strain SMH4021, GenBank AY780059.1; Identities = 827/833 (99%), 1 gap (0%)), and the closest hit using the TUB sequence was *Cladorrhinum foecundissimum* (strain CBS 391.42, GenBank HQ877462.1; Identities = 282/300 (94%), 3 gaps (1%)).

Cladorrhinum yunnanense formed a well-supported sister clade (BS = 99%, PP = 1.0) to *C. flexuosum* and *C. samala*. Based on conidial

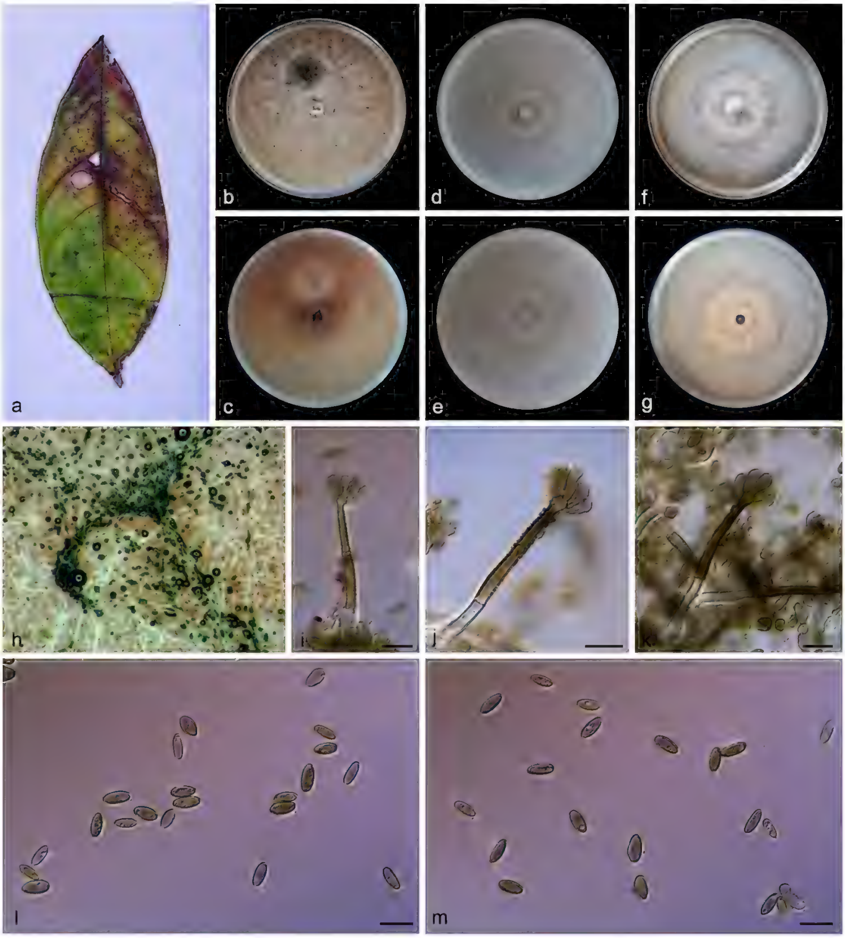

FIG. 2. *Cladorrhinum yunnanense* (holotype, HSAUP 0525). a. Diseased leaf; b,c. Colony on PDA; d,e. Colony on OA; f,g. Colony on MEA; h. Conidiomata; i-k. Conidiophores and conidia; l-m. Conidia. Scale bars: i-m = 10 µm

morphology, *C. yunnanense* (oval to ellipsoid, 6–10 × 2–4 µm) can be distinguished from *C. flexuosum* (mostly globose to dacryoid, 2–4 × 2–3 µm) and *C. samala* (dacryoid, 2–4 × 1.5–3 µm). *Cladorrhinum yunnanense* also differs from the previously described *Cladorrhinum* species by its blastic conidiogenous cell.

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